Journal of Research on the Lepidoptera 19(2): 82-87, 1980(81)

Canalization of the Phenotype of *Nymphalis antiopa* (Lepidoptera: Nymphalidae) from Subarctic and Montane Climates

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Abstract. *Nymphalis antiopa* from the montane Sierra Nevada of California respond phenotypically to pupal cold shock in a manner similar to lowland California *antiopa* and unlike subspecies *hyperborea* from Alaska. This could result from historical factors, gene flow, or canalization of the phenotype in the Sierran climate, which shows very wide diel temperature fluctuations but not the prolonged cool spells common in the Alaskan summer.

The concept of canalization of development (Waddington, 1957) has been thoroughly assimilated into the evolutionary framework of modern biology. Like many other biological concepts deriving from seemingly impregnable Darwinian deduction, it has rarely been tested experimentally; although physiological adjustment in geographic populations is routinely demonstrated, developmental buffering is not. Shapiro (1981) attempted to test the hypothesis that canalization is adaptive to the physical environment, using the phenotypic response of the Mourning Cloak butterfly, *Nymphalis antiopa* (Linnaeus) to pupal cold shock. It has been known for over a century that various species of the tribe Nymphalini respond to temperature shock by producing predictable, often very radical, pattern aberrations. In the most dramatic aberration of *N. antiopa*, "hygiaea," the yellow border is doubled in width, and the blue spots normally arranged in a row basad of it are entirely absent (Fig. 1). "Hygiaea" can be induced by a variety of treatments (Fischer, 1907; Standfuss, 1896). One treatment which is very efficacious at inducing it in near-sea-level Californian stock is exposure of 8-hr-old pupae to 2°C for 2 weeks. Shapiro (1981) reasoned that the subarctic subspecies *N. a. hyperborea* (Seitz), which has a relatively high probability of being chilled in the pupal stage, should be better buffered developmentally against cold than lowland Californian *N. a. antiopa*, which are assured of benign conditions in a Mediterranean climate. Treatment failed to produce any "hygiaea" among 53 Alaskan animals, vs. 31 among 157 lowland Californian ones. This is a highly significant difference ($\chi^2 = 12.28$, $p < 0.005$). Limitations on the interpretation of these results are spelled out by Shapiro (1981).

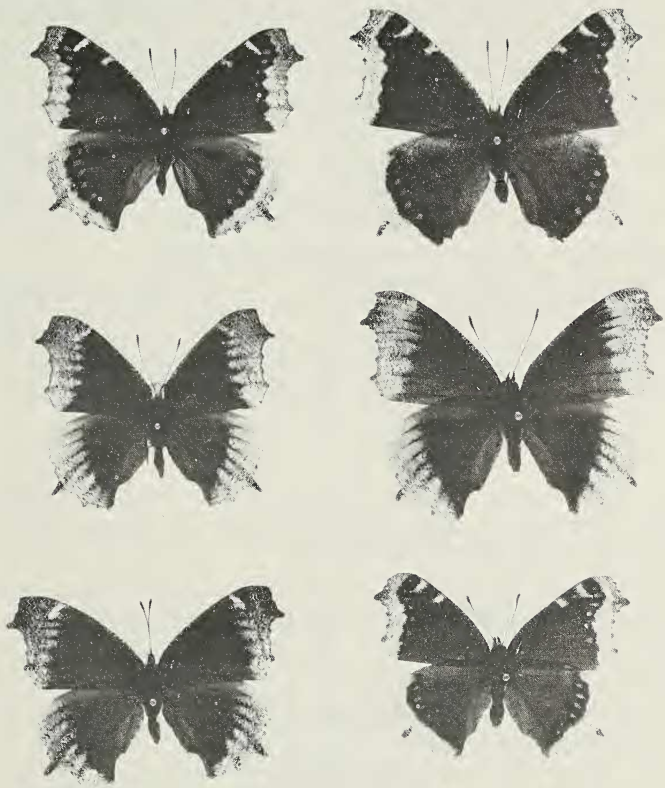

Fig. 1. Bred, cold-shocked *Nymphalis antiopa*. Left: Donner Pass, Sierra Nevada, normal (top) and "hygiaea" (center, brood 3; bottom, brood 4). Right: Vacaville, Solano County, lowland California, normal (top) and "hygiaea" (center); ssp. *hyperborea*, Fairbanks, Alaska, 1979 (bottom).

N. antiopa occurs in California from sea-level to tree-line. Populations at high altitudes, like Alaskan ones, are likely to be chilled during development. The pattern of probable exposure differs radically between the two areas, however. Alaskan *hyperborea* are larvae in June-early July and pupae in mid-July (Fairbanks). They are likely to experience periods of cool, showery weather with temperatures between 40-50°F), but no frost. Such weather is almost impossible in the Sierra Nevada of California. At 7000 ft. (2100^{+} m), *antiopa* are larvae in late July-early August, and pupae in mid-August. Afternoons are clear to partly cloudy with frequent highs of

80°F (27°C), but nocturnal freezes are very common. Thus, Alaskan *antiopa* may develop in prolonged cool but not cold conditions, while Sierran ones are cold almost every night but warm almost every day. Summer monthly mean temperatures for Fairbanks, Alaska and Truckee, California are similar, but the diel ranges are very different (Tables 1, 2). Since short exposures (less than 1 wk) to 2°C have little or no phenotypic effect on *N. antiopa*, and the full 2-wk exposure is required to obtain the full expression of "hygiaea," it would not be surprising if Sierran *antiopa* were less well buffered against sustained low temperatures than Fairbanks *hyperborea*. This was tested experimentally in 1980.

Five colonies of larvae were collected in the 3rd instar on willows (*Salix* spp.) at two Sierran localities: one from Martis Creek, near Truckee, Placer Co. (elevation about 5600 ft. = 1707 m), July 5, 1980, and four from the east end of Donner Pass along U. S. Highway 40 east of Norden, Placer Co. (7000 ft. = 2134 m), August 7, 1980. Martis Creek is a sedgy swale

Table 1. Comparisons of summer temperatures for Fairbanks, Alaska and Truckee, California for a sample year (1977). Data from National Oceanic and Atmospheric Administration, Asheville, N. C.

VALUES IN °F AND (IN PARENTHESES) °C

Variable	Fairbanks[1]			Truckee[2]		
	June 77	July 77	Aug. 77	June 77	July 77	Aug. 77
mean daily maximum	68.8 (20.4)	73.7 (23.1)	73.2 (22.9)	77.4 (25.2)	80.7 (27.0)	81.6 (27.5)
mean daily minimum	50.4 (10.2)	51.9 (11.0)	52.0 (11.1)	42.1 (5.6)	41.8 (5.4)	44.5 (6.9)
mean daily temperature	59.6 (15.3)	62.8 (17.1)	62.6 (17.0)	59.8 (15.4)	61.3 (16.3)	63.1 (17.3)
departure of mean daily T from long-range mean daily T	+0.6 (+0.3)	+2.1 (+1.2)	+7.2 (+4.0)	+6.3 (+3.5)	0 0	+3.4 (+1.9)
number of nights with temperature below 40°F (4.4°C)	0	0	2	10	12	6

[1]Fairbanks Airport, 64°49′ N, 147°52′ W, elevation 436 ft. = 182.9 m.
[2]Truckee Ranger Station, 39°20′ N, 120°11′ W, elevation 5995 ft. = 1827.3 m.

Table 2. Freeze data for Fairbanks, Alaska and Truckee, California. Station data as in Table 1. Source: NOAA.

	DATE OF LAST SPRING OCCURRENCE OF:				
	16°F (-8.9°C)	20° (-6.7)	24° (-4.4)	28° (-2.2)	32° (0)
Fairbanks 1977	16.IV	29.IV	29.IV	29.IV	10.V
Truckee 1977	19.V	19.V	28.V	29.V	11.VI
	DATE OF FIRST FALL OCCURRENCE OF:				
	32°F (0°C)	28° (-2.2)	24° (-4.4)	20° (-6.7)	16° (-8.9)
Fairbanks 1977	4.IX	1.X	1.X	13.X	16.X
Truckee 1977	5.VII	21.IX	22.IX	7.XI	9.XI
	NUMBER OF DAYS BETWEEN LAST SPRING AND FIRST FALL OCCURRENCE OF:				
	16° (-8.9)	20° (-6.7)	24° (-4.4)	28° (-2.2)	32° (0)
Fairbanks 1977	183	167	155	155	117
Truckee 1977	174	172	117	115	24

running through Great Basin shrub desert (*Artemisia, Purshia*). The Donner site is mesic-montane and receives a very heavy winter snowpack. Both populations are univoltine. The two sites are about 12 mi (19.3 km) apart. Martis Creek receives cold air drainage at night and is both hotter by day and colder by night than Donner Pass. Truckee Ranger Station (5995 ft. = 1827 m) provides a weather station intermediate in climate and elevation between them.

The Martis Creek colony was reared to maturity on English Elm, *Ulmus procera* (Ulmaceae). This tree became unavailable by August due to defoliation by beetle larvae, and the four Donner broods were reared instead on Hackberry, *Celtis sinensis* (Ulmaceae). All rearing was under continuous light at 25°C. Eight-hr pupae were refrigerated in the dark at 2°C, held for 14 days, and returned to 25°C. Larval mortality was negligible in all broods.

Pupal mortality was very heavy in refrigerated groups from all broods, and exceeded 75% overall. Such heavy mortality is unusual. Moreover, chilled animals usually die as pharate adults, which can be scored for phenotype; in these broods most mortality occurred before wing pigmentation was laid down. *Celtis* is an unusual host, but the very poor survivorship of the Martis Creek brood, reared on *Ulmus*, argues against it as a major cause of pupal mortality; it seems that Sierran *antiopa* are poorly protected physiologically against long chilling in the pupa, even compared to lowland Californian ones.

Results and Discussion

Phenotypic sensitivity paralleled pupal mortality: seven "hygiaea" were produced among 61 scorable Donner animnals (Table 3). This is not significantly different from 31/157 from lowland California ($\chi^2 = 1.72$, $0.250 > p > 0.100$) but is more unlike 0/53 from Alaska ($\chi^2 = 6.23$, $0.025 > p > 0.010$). Most suggestively, all four Donner broods gave at least one "hygiaea" each, just as 12/13 lowland Californian broods reared in past years have done. Neither of the 1979 Alaskan broods produced even one individual transitional to "hygiaea." The very small Martis Creek emergence does not significantly affect the statistical comparisons.

Alaskan *hyperborea* exaggerate their subspecific characters when chilled. The same modifications were seen in both Martis and Donner *antiopa*. They were often quite marked in animals scored "normal" for purposes of this analysis. Sierran animalss also resembled Alaskan ones in size (mean LFW: lowland CA, 37 mm; Sierra Nevada, 30.3 mm; Fairbanks, 29.0 mm). Unlike *hyperborea*, Sierran experimentals often showed slight modifications in the direction of "hygiaea"; almost all of Donner brood 3 showed them. Several other odd phenotypes were obtained in the chilled Sierran *antiopa*: one animal each in Donner 1 and Martis had the wings thinly scaled, and the Martis individual also had the blue spots oddly shaped and arranged, and of a violet color. One individual in Donner 2 had the hindwings symmetrically falcate.

This variability in chilled Sierran *antiopa* contrasts with both the unchilled controls and with wild-collected material. In a series of about 3 dozen from Donner Pass, Truckee, and nearby Sagehen Creek the phenotype is quite uniform: the size is larger (mean LFW 34.5 mm), there is a slight increase in black in the borders relative to lowland specimens, and the blue spots may be slightly enlarged, especially on the hindwing. The increased black scaling in the borders is duplicated in both control and experimental Sierran broods, increased in the latter. Certain other temperature treatments favor increased blue spotting in both European (Standfuss, 1896) and lowland Californian (Shapiro, unpublished) *antiopa*.

Sierran *antiopa* thus behave like lowland Californian ones with respect to the major aberration "hygiaea," but in other responses to chilling they are intermediate in response between lowland Californian and Alaskan broods, and more variable than either. The "hygiaea" response can be accounted for in at least three ways. Two of these imply lack of adaptation to local climate: phylogenetic inertia (recent common ancestry of lowland and Sierran *antiopa*) and gene flow (constant or intermittent). There are no data bearing directly on either. The species is highly dispersive, and occurs locally from the coast across the Sierra in riparian habitats. Differences in the phenotypes of chilled lowland and Sierran animals argue for some degree of differentiation among the populations. The third hypothesis specifically invokes local climatic adaptation. The dissimilarity

Table 3. Results of temperature-shock experiments with *Nymphalis antiopa* from the Sierra Nevada of California.

Brood, source	**Normal live**	**Normal dead**	**Hygiaea live**	**Hygiaea dead**	**Unscorable dead**	**Total**
Donner Pass #1						
experimental	18	0	1	0	53	72
control	10	0	0	0	0	10
Donner Pass #2						
experimental	18	1	2	1	45	67
control	10	0	0	0	0	10
Donner Pass #3						
experimental	14[1]	1	2	0	57	74
control	10	0	0	0	0	10
Donner Pass #4						
experimental	2	0	1	0	23	26
control	8	0	0	0	2	10
Martis Creek						
experimental	2[2]	4	0	0	11	17
control	5	0	0	0	0	5
Totals						
experimental	54	6	6	1	189	256
control	43	0	0	0	2	45
Total Reared:						**301**

[1]most individuals show definite "hygiaea" tendencies.
[2]includes one thinly scaled and otherwise aberrant animal (see text).

of Alaskan and Sierran animals could reflect the different temperature regimes and local adaptation to them, but until considerably more is known of the ecology and genetics of this butterfly it is not possible to distinguish among these three hypotheses. Despite its abundance, the Mourning Cloak is rather poorly known biologically (Young, 1980).

Acknowledgements: I thank Marc Minno, Michael Collins, and James W. Beever III for assistance and Wanda Reynolds and Carol Erickson for discussion.

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